

In the Specification:

Please amend the specification as shown:

Please delete the paragraphs on page 11, line 1 to page 12, line 2 and replace them with the following paragraphs:

In a preferred embodiment of the invention the peptide tag may be an epitope, that is a defined amino acid sequence from a protein with a fully characterised cognate antibody. The skilled person can select such epitopes based on sequences identified as possessing antigenic properties. In certain embodiments of the invention the epitope tag may be the amino acid sequence below from the c-myc oncogene (Evans *et al Mol. Cell. Biol.* 5 3610-3616 (1985)):

-Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu- **(SEQ ID NO: 1)**

(EQKLISEEDL) **(SEQ ID NO: 1)**

or it may be the amino acid sequence from the simian virus V5 protein (Southern *et al J. Gen. Virol.* 72 1551-1557 (1991)), shown below:

-Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr- **(SEQ ID NO: 2)**

(GKPIPNPLLGLDST) **(SEQ ID NO: 2)**

In certain embodiments of the invention, the epitope may be selected from but not limited to the c-myc and V5 proteins.

Other alternative epitopes may include, but are not limited to:

Haemagglutinin	(YPYDVDPDYA) (<u>SEQ ID NO: 3</u>)
Clone100	(NVRFSTIVRRRA) (<u>SEQ ID NO: 4</u>)
rab11a	(KQMSDRRENDMSPS) (<u>SEQ ID NO: 5</u>)
DOB	(SGNEVSRAVLLPQSC) (<u>SEQ ID NO: 6</u>)
SG11	(SSLSYTNPAVAATSANL) (<u>SEQ ID NO: 7</u>)
erbB4	(RSTLQHPDYLQEYST) (<u>SEQ ID NO: 8</u>)
ARF	(VSTLLRWERFPGHRQA) (<u>SEQ ID NO: 9</u>)
RYK	(KFQQLVQCLTEFHAALGAYV) (<u>SEQ ID NO: 10</u>)
WILPEP1	(QEQCQEVWRKRVISAFKSP) (<u>SEQ ID NO: 11</u>)
HAF10	(RLSDKTGPPVAQEKS) (<u>SEQ ID NO: 12</u>)

Please delete the paragraphs on page 34, lines 18-31 and replace them with the following paragraphs:

FIGURE 7 shows the DNA (SEQ ID NO: 13) and amino acid (SEQ ID NO: 14) sequences of the MUP clone Mmup9a. The 18 amino acid secretion signal peptide is shown in bold (amino acid residues 1 to 18).

FIGURE 8 shows the DNA (SEQ ID NO: 15) and amino acid (SEQ ID NO: 16) sequence of the recombinant mMUP reporter molecule. The protein contains a sixteen amino acid N-terminal addition, comprising of 6 amino acids from the pGEX vector (italics – amino acid residues 1 to 6) and the c-myc epitope (shown in bold – amino acid residues 7 to 16).

FIGURE 9 shows the DNA (**SEQ ID NO: 17**) and amino acid (**SEQ ID NO: 18**) sequence of the recombinant BLGm reporter molecule. The protein contains a six amino acid N-terminal addition from the pGEX vector (*italics* – amino acid residues 1 to 6) and the C-terminal c-myc epitope (**bold** – amino acid residues 170 to 179).

Please delete the paragraph on page 36, lines 9-14 and replace it with the following paragraph:

FIGURE 15 shows modified MUP proteins produced from the pSecTag vector (**SEQ ID NOS: 19-22, respectively, in order of appearance**). The various modifications made to the wild-type MUP protein sequence (overlined region) are shown: the Igk signal peptide leader, which is cleaved during processing (++++); the c-myc epitope tag (underlined); the iTag insertion sequence in the FG loop (*italics*); and the Clone 100 epitope tag (**bold**), and the other C- and N-terminal modifications and additions.

Please delete the paragraphs on page 36, line 30 to page 38, line 10 and replace them with the following paragraphs:

FIGURE 18 shows the nucleotide sequence for ovine betalactoglobulin (BLG) (**SEQ ID NO: 23**; accession no. X12817), available from www.ncbi.nlm.nih.gov/entrez, published by Harris, S *et al Nucleic Acids Res.* **16** (21), 10379-10380 (1988); Watson, C.J. *et al Nucleic Acids Res.* **19** (23), 6603-6610 (1991). The signal peptide is coded for by residues 842 to 895 and mature protein from 6 exons at residues 896..937,1602..1741,2586..2659,3772..3882,4551..4655, 4869..4882

FIGURE 19 shows the amino acid sequence for ovine betalactoglobulin (BLG) (**SEQ ID NO: 24**) coded for by the nucleotide sequence of Figure 16.

FIGURE 20 shows the cDNA encoding the mRNA of murine major urinary protein 1 (Mup1), (**SEQ ID NO: 25**; Accession no. NM 031188), available from www.ncbi.nlm.nih.gov/entrez, published Lucke *et al Eur. J. Biochem.* **266** (3), 1210-1218 (1999); Abbate, *et al J. Biomol. NMR* **15** (2), 187-188 (1999); Ferrari *et al FEBS Lett.* **401** (1), 73-77 (1997); Held, *et al Mol. Cell. Biol.* **7** (10), 3705-3712 (1987); Bennett *et al J. Cell Biol.* **105** (3), 1073-1085 (1987); Shahan *et al Mol. Cell. Biol.* **7** (5), 1938-1946 (1987); Clark *et al EMBO J.* **4** (12), 3167-3171 (1985); Clark, *et al EMBO J.* **4** (12), 3159-3165 (1985); Ghazal *et al Proc. Nat'l. Acad. Sci. USA.* **82** (12), 4182-4185 (1985); Kuhn *et al Nucleic Acids Res.* **12** (15), 6073-6090 (1984); Clark *et al EMBO J.* **3** (5), 1045-1052 (1984); Krauter *et al J. Cell Biol.* **94** (2), 414-417 (1982); coding sequence from residues 112..654.

FIGURE 21 shows the amino acid sequence for murine major urinary protein (**SEQ ID NO: 26**) coded for by the nucleotide sequence of Figure 18.

FIGURE 22 shows the cDNA sequence encoding the mRNA of rat alpha-2-u globulin (**SEQ ID NO: 27**; accession no. M27434), available from www.ncbi.nlm.nih.gov/entrez, published by Roy *et al J. Steroid Biochem.* **27** (4-6), 1129-1134 (1987)

FIGURE 23 shows the GST coding sequence derived from pGEX6p-1. The GST coding sequence is nucleotide residues 241-917. The residues highlighted in bold

Leu	Glu	Val	Leu	Phe	Gln	Gly	Pro (<u>SEQ ID NO: 32</u>)
ctg	gaa	ggt	ctg	ttc	cag	ggg	ccc (<u>SEQ ID NO: 31</u>)

represent the PreScission™ Protease cleavage recognition sequence position 918-938. The protease cleavage site allows for the production of cleaved myc-tagged proteins from the GST fusion proteins as described in Example 6. **Nucleotide sequences disclosed as SEQ ID NOS: 28-29, respectively, in order of appearance. Protein sequence disclosed as SEQ ID NO: 30.**

Please delete the paragraphs on page 43, line 20 to page 45, line 22 and replace them with the following paragraphs:

MUP and BLG lipocalin reporter proteins have been successfully tagged with N- and C-terminal tags (above data for GST and c-myc tags). Internal loop positions within the MUP protein have also been used to introduce the peptide epitope sequences. Several potential positions for the introduction of epitope tags were chosen, from the MUP protein structure (Figure 15), as being in external loops. The initial position chosen to introduce a tag corresponded to a site within the EF loop of BLG protein that had previously been used to introduce a kinase recognition site. This had utilised a *Clal* restriction site in the BLG gene, however there is no corresponding restriction site in the MUP gene. Consequently, the Mup cDNA sequence was modified by the introduction of a) an *AvrII-ApaI-SbfI* linker fragment into the sequence coding for EF loop region and b) a *SpeI-EcoRI-NsiI* linker fragment at the 3' end of the coding sequence. The particular restriction site combinations were chosen since they would generate compatible overhanging ends, for the insertion of adapter oligonucleotides containing epitope sequences. The MUP 5'-coding region from position 10 to 300, together with an additional GATGCGGTACCACCATGGTGTCTAGACTGCAG (**SEQ ID NO: 33**) 5'-sequence (containing a Kozak signal, start codon and *NcoI-KpnI-XbaI-PstI* linker) and an additional CCTAGGC sequence (containing an *AvrII* restriction site) was generated by PCR. The corresponding MUP 3'-region from position 301 to 540, together with an additional TGCCTAGGGCCCTGCAGGGTA (**SEQ ID NO: 34**) 5'-sequence (containing an *AvrII-ApaI-*

SbfI linker) and ACTAGTGAATTCATGCATTGAGCTAGCCATC (**SEQ ID NO: 35**) 3'-sequence (containing an SpeI-EcoRI-NsiI-NheI linker and stop codon) was generated by PCR. Ligation of these two fragments, at the common AvaII site generated the required modified MUP coding sequence, on a NcoI-NheI fragment.

Restriction digest with either AvrII/SbfI (internal EF loop) or SpeI/NsiI (C-terminus) results in an identical pattern of overhanging ends, to which double stranded oligonucleotide linkers, of the general form:

CTAG N (NNN)_x N TGCA (**SEQ ID NO: 36**)

N (NNN)_x

where x is a multiple of 3, that contain an epitope tag, can anneal.

MUP lipocalin reporter proteins have also been produced, in which the epitope has been introduced into the FG loop position. This has been accomplished by the insertion of a *HindIII-BamHI-EcoRI* linker fragment into the MUP coding sequence at the FG loop position. This has allowed the insertion of adapter oligonucleotides containing epitope sequences into the *HindIII/EcoRI* sites. The MUP coding sequence, from position 1 to 348, together with an additional GGTACCACC 5'-sequence (containing a KpnI restriction site and Kozak sequence) and an additional AAGCTTGGAACCGGATCC (**SEQ ID NO: 37**) 3'-sequence (containing *HindIII-BamHI* sites) was generated by PCR, as was the corresponding MUP coding sequence from position 349 to 540, together with an additional GGATCCTCTTCAGAATTC (**SEQ ID NO: 38**) 5'-sequence (containing *BamHI* and *EcoRI* restriction sites) and an additional GAGCAGAACTCATCTCTGAAGAGGATCTGTGAGCTAGC (**SEQ ID NO: 39**) 3'-sequence (containing the c-myc GluGlnLysLeuIleSerGluGluAspLeu (**SEQ ID NO: 1**) epitope tag, stop codon and *NheI* restriction site). Ligation of the two fragments, at the *BamHI* site generated the modified MUP coding sequence, on a NcoI-NheI fragment.

Restriction digest with HindIII/EcoRI results in overhanging ends, to which double stranded oligonucleotide linkers, of the general form:

AGCT T (NNN)_x G (**SEQ ID NO: 40**)

A (NNN)_x C TTAA (**SEQ ID NO: 41**)

where x is a multiple of 3, that contain an epitope tag, can anneal.

Epitopes that have been inserted into the FG loop, by this method, include:

Haemagglutinin	(YPYDVPDYA) (<u>SEQ ID NO: 3</u>)
Clone100	(NVRFSTIVRRRA) (<u>SEQ ID NO: 4</u>)
rab11a	(KQMSDRRENDMSPS) (<u>SEQ ID NO: 5</u>)
DOB	(SGNEVSRAVLLPQSC) (<u>SEQ ID NO: 6</u>)
SG11	(SSLSYTNPAVAATSANL) (<u>SEQ ID NO: 7</u>)
erbB4	(RSTLQHPDYLQEYST) (<u>SEQ ID NO: 8</u>)
ARF	(VSTLLRWERFPGHRQA) (<u>SEQ ID NO: 9</u>)
RYK	(KFQQLVQCLTEFHAALGAYV) (<u>SEQ ID NO: 10</u>)
WILPEP1	(QEQCQEVWRKRVISAFKSP) (<u>SEQ ID NO: 11</u>)
HAF10	(RLSDKTGPVAQEKs) (<u>SEQ ID NO: 12</u>)